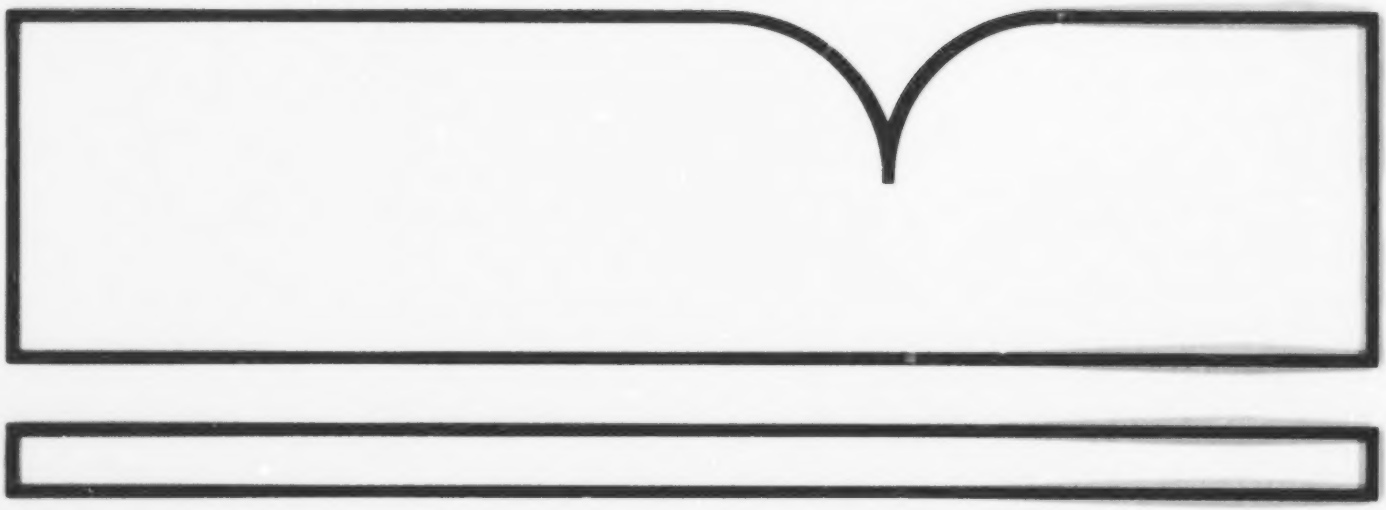
PB86-118866

Evaluation of the Immunotoxic Potential of Chlordecone with Comparison to Cyclophosphamide

(U.S.) Health Effects Research Lab.
Research Triangle Park, NC

1985



U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA/600/J-85/200	2.	3. PB86-118866
4. TITLE AND SUBTITLE EVALUATION OF THE IMMUNOTOXIC POTENTIAL OF CHLORDECONE WITH COMPARISON TO CYCLOPHOSPHAMIDE (Journal Version)		5. REPORT DATE 1985 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) R.J. Smialowicz, R.W. Luebke, M.M. Riddle, R.R. Rogers, and D.G. Rowe		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS same as #12		10. PROGRAM ELEMENT NO. ACSL1A 11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Health Effects Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Research Triangle Park, NC 27711		13. TYPE OF REPORT AND PERIOD COVERED 14. SPONSORING AGENCY CODE EPA/600/11

15. SUPPLEMENTARY NOTES

REF: Journal of Toxicology and Environmental Health, 15:561-574, 1985

16. ABSTRACT

The immunotoxic potential of chlordecone was evaluated in male Fischer 344 rats following 10 days of dosing by oral gavage. These results were compared with a comparable dosing regimen with the known immunosuppressive drug cyclophosphamide. Significant changes in the immune parameters examined, which included spleen and thymus to body weight ratios, mitogen responsiveness to the lymphocyte mitogen conconavalin A and natural killer (NK) cell activity against allogeneic W/Fu-G1 rat lymphoma target cells and xenogeneic YAC-1 mouse lymphoma target cells were observed only at the highest dosage (10 mg/kg/day). A significant decrement in body weight also occurred at this dosage which suggests that the observed changes in the immune parameters measured were most likely due to the overt toxicity of chlordecone at this dosage. Rats dosed over 10 days by oral gavage with cyclophosphamide showed significant decreases in spleen and thymus to body weight ratios at a dosage as low as 1.5 mg/kg/day. Body weight decrements were observed at dosages of 12 mg/kg/day or greater. At this same dosage of cyclophosphamide significant decreases were observed for the following: 1) total leukocyte and absolute lymphocyte counts; and 2) lymphoproliferative responses to both T- and B-lymphocyte mitogens. Significant suppression of NK cell activity was observed at dosages of 6 mg/kg/day or greater. It appears that T and B lymphocytes are more sensitive than are NK lymphocytes to the immunosuppressive effects of cyclophosphamide. These results indicate that chlordecone did not affect the immune parameters examined in the absence of overt toxicity.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS *(This Report)* UNCLASSIFIED	21. NO. OF PAGES 15
	20. SECURITY CLASS *(This page)* UNCLASSIFIED	22. PRICE

EPA/600/J-85/206
JOURNAL ARTICLE

EVALUATION OF THE IMMUNOTOXIC POTENTIAL OF CHLORDECONE WITH COMPARISON TO CYCLOPHOSPHAMIDE

Ralph J. Smialowicz, Robert W. Luebke, Marie M. Riddle, Ronald R. Rogers, Denise G. Rowe

Immunobiology Section, Developmental Biology Division, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina

The immunotoxic potential of chlordecone was evaluated in male Fischer-344 rats following 10 d of acute dosing by oral gavage. These results were compared with those obtained following a comparable dosing regimen with the known immunosuppressive drug cyclophosphamide. Significant changes in ratios of spleen and thymus to body weight, blastogenic responsiveness of lymphocytes to concanavalin A, and natural killer (NK) cell activity against allogeneic W/Fu-G1 rat lymphoma target cells and xenogeneic YAC-1 mouse lymphoma target cells were observed only at the highest chlordecone dosage (10 mg/kg•d). A significant decrement in body weight also occurred at this dosage which suggests that the observed changes in the immune parameters measured were most likely due to the overt toxicity of chlordecone. In contrast, rats dosed over 10 d by oral gavage with cyclophosphamide showed significant decreases in spleen and thymus to body weight ratios at a dosage as low as 1.5 mg/kg•d. Body weight decrements were observed only at dosages of 12 mg/kg•d or greater. At dosages of 1.5 mg/kg•d or greater, cyclophosphamide caused a significant decrease in the total leukocyte and absolute lymphocyte counts in peripheral blood, and a decrease in the lymphoproliferative responses to both T- and B-lymphocyte mitogens. Significant suppression of NK cell activity was observed at dosages of 6 mg/kg•d or greater. These results suggest that T and B lymphocytes are more sensitive than are NK lymphocytes to the immunosuppressive effects of cyclophosphamide. The results of these two studies indicate that the failure to detect chlordecone-induced immunotoxic effects in the absence of overt toxicity is not due to the possible insensitivity of the parameters examined. Instead, since cyclophosphamide affected all of the parameters tested, these results indicate that the endpoints examined here are capable of detecting immunotoxicity with sufficient sensitivity to permit distinction between probable indirect immunosuppression due to overt toxicity and subtle, direct impairment of the immune system.

This paper has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and is approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

The following individuals are recognized for their contribution to the successful completion of this study: R. J. Garner, J. Lewtas, B. M. Andon, L. L. Hall, E. Berman, B. Edwards, F. Poythress, and F. Gregory.

Requests for reprints should be sent to Ralph J. Smialowicz, MD-67, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711.

Journal of Toxicology and Environmental Health, 15:561–574, 1985

INTRODUCTION

Chlordecone (decachlorotetracyclodecanone), commercially known as Kepone, is a polycyclic chlorinated insecticide that has been used to control tobacco wireworm, banana root borer, ants, and roaches. This insecticide, which has been removed from the market in the United States because of its suspected and proven toxicity (Baggett et al., 1980), is lipophilic and is very persistent in the environment (Metcalf et al., 1973). It has been reported that chlordecone, which also exists as a degradation product of mirex, can be found as a major toxicant in soil several years after initial application of mirex (Carlson et al., 1976).

Some of the sequelae of human exposure to chlordecone during manufacture of this pesticide include liver damage, sterility in males, and neuropathological symptoms (Cohn et al., 1976). Of the various clinical symptoms of chlordecone exposure, the most severe and rapidly developing effects in experimental animals are tremors and other signs of neurological impairment (Egle et al., 1979, Gerhart et al., 1983; Seth et al., 1981). Tremors have also been reported to occur in workers who were exposed to chlordecone during production of this pesticide (Taylor et al., 1978).

In addition to neurological disorders, chlordecone has been shown to be fetotoxic in rats and mice (Chernoff and Rogers, 1976), to have estrogenic-like effects in mice and quail (Eroschenko and Mousa, 1979; Eroschenko and Wilson, 1975; McFarland and Lacey, 1969), and to affect the reproductive systems in mice (Good et al., 1965; Huber, 1965) and in chickens (Naber and Ware, 1965). Chlordecone has also been reported to be carcinogenic in mice and rats by the National Cancer Institute (Anonymous, 1976).

Despite the many toxicologic studies of chlordecone in experimental animals, we are unaware of any published studies that have examined the immunotoxic potential of this insecticide. The estrogenic-like properties and potential carcinogenicity of this chemical makes it a good candidate for immunotoxicity testing because of the known effects of estrogenic compounds on the immune system (Slijivic and Warr, 1973; Ahlquist, 1976; Luster et al., 1979) and the fact that some carcinogens are potent immunomodulators (Baxter et al., 1981, Stevens and Cole, 1982).

The purpose of this investigation was to determine if repeated dosing (10 consecutive days) of rats with chlordecone would affect the immune system. Various parameters of immune system integrity were examined in these animals. The effects of the known immunosuppressive drug cyclophosphamide (Turk and Poulter, 1972) were also determined for these same parameters. The effects of cyclophosphamide served to demonstrate the sensitivity of the immune parameters investigated.

This study was conducted as part of an effort by the Health Effects Research Laboratory of the U.S. Environmental Protection Agency (EPA) to develop a panel of tests to assess the potential health effects of complex hazardous waste mixtures present in the environment. Several biological

systems, including the immune system, are being examined as potential endpoints in this screen. The results presented here represent the initial steps using a series of pure compounds (e.g., cyclophosphamide) with known biological effects toward the design, validation, and selection of a protocol that can be applied to assess complex waste mixtures which are found in the environment. Chlordecone was chosen as a test compound because of its known toxicity. It also represents a pesticide which persists for long periods of time in the environment (Carlson et al., 1976).

METHODS

Animals

Male Fischer-344 rats were received in our vivarium at 60 d of age from Charles River Labs, Kingston, N.Y. Rats were housed two per cage in shoebox cages with heat-treated pine shavings, and received food (Purina Rodent Lab Chow, 500-1, Richmond, Ind.) and tap water ad libitum. The animal room was maintained at a relatively constant temperature (22 ± 2°C) and humidity (50 ± 10%) with a 12 h light–dark cycle. At 9 d after arrival, rats were weighed, identified with an ear tag, and randomly assigned to one of 7 dose groups with 10 rats/dose. Dose groups included five concentrations of test material and two controls (cage and vehicle).

Exposures

Rats were dosed by oral gavage in the morning on 10 consecutive days. The dose was given on a body weight basis with doses calculated for each rat's weight. Rats were weighed at 3-d intervals. Technical grade chlordecone (87% pure, U.S. EPA Pesticide and Industrial Chemical Repository, Research Triangle Park, N.C.) was dissolved in corn oil and administered to rats at doses of 0.625, 1.25, 2.5, 5.0, and 10.0 mg/kg·d. Cyclophosphamide (Sigma Chemical Co., St. Louis, Mo.) was dissolved in distilled water and administered to rats at doses of 1.5, 3.0, 6.0, 12.0, and 24.0 mg/kg·d.

Immune Parameters

At 48 h after the tenth and final dosing, animals were weighed, anesthetized with ether, and bled from the abdominal aorta. Total leukocyte counts and differential (percent) counts were performed on blood samples. The spleen and thymus were aseptically removed and weighed.

The proliferative response of splenic lymphocytes was determined for the T-cell mitogens phytohemagglutinin (PHA-P; Burroughs-Wellcome, Research Triangle Park, N.C.) and concanavalin A (Con A; Difco Lab., Detroit, Mich.), the B-cell mitogen *Salmonella typhimurium* mitogen (STM; Ribi Immunochem, Hamilton, Mont.); and the T- and B-cell mitogen pokeweed mitogen (PWM; Grand Island Biological Co., Grand Island, N.Y.). Spleen-cell cultures were prepared in RPMI-1640 medium supplemented

with 10% heat-inactivated fetal bovine serum (FBS), 25 m*M* HEPES, gentamycin at 50 μg/ml, and 5×10^{-5} *M* 2-mercaptoethanol (2-ME) according to Oehler and Herberman (1978). Lymphocytes were cultured at 5×10^5 splenocytes/well in 96-well flat-bottom culture plates (Costar, Cambridge, Mass.) for 3 d. Cultures were labeled with tritiated thymidine ([^{3}H]TdR, 0.5 μCi/well, specific activity 6.7 Ci/m*M*, New England Nuclear, Boston, Mass.) 4 h prior to harvest on a MASH II automated harvester (Microbiological Associates, Bethesda, Md.). Lymphoproliferative responses were determined in triplicate at three concentrations of each mitogen. The optimal responses for each mitogen are reported (i.e., 0.5 μg/culture of PHA and Con A, 10 μg/culture of PWM, and 4 μg/culture of STM).

Natural killer (NK) cell activity of rat splenocytes, cultured in supplemented RPMI without 2-ME, was determined as described by Oehler and Herberman (1978). W/Fu-G1 rat lymphoma cells (obtained from Dr. Craig Reynolds, NCI, Bethesda, Md.) and YAC-1 mouse lymphoma cells (obtained from Dr. Ann Burger, Duke University, Durham, N.C.), free of mycoplasma, were used as target cells in this study. ^{51}Cr-labeled target cells (2×10^4/well were cocultured in triplicate with effector cells (splenocytes) for 4 h in a volume of 200 μl in round-bottom microtiter plates (Linbro) at effector:target cell (E:T) ratios of 25:1, 50:1, and 100:1. After incubation at 37°C in a humidified atmosphere of 5% CO_2 and 95% air, the plates were centrifuged for 5 min at 250 × g, and the supernatant was collected using the Titertek supernatant collection system (Flow Labs, Rockville, Md.). Percent specific ^{51}Cr release was determined from the formula:

$$[(E - S)/(T - S)] \times 100$$

In this formula E is the ^{51}Cr released from target cells in the presence of spleen cells, S is the spontaneous release of ^{51}Cr from target cells alone, and T is the maximum release of ^{51}Cr from target cells in the presence of 0.25% Triton X-100. Spontaneous release was typically less than 10% of the maximum releasable ^{51}Cr activity.

Statistical Analysis

Data were analyzed using Dunnett's *t*-test with all comparisons made against cage control values. No differences were observed between cage and vehicle control data for all of the parameters studied.

RESULTS

Tables 1 and 2 show the lymphoid organ and body weight data for chlordecone and cyclophosphamide dosed rats, respectively. A significant ($p < 0.01$) reduction in spleen and thymus weight was observed only at the highest dosage (10 mg/kg•d) of chlordecone (Table 1). Overt toxicity

TABLE 1. The Effect of Chlordecone on Rat Lymphoid Organ and Body Weights

Treatment (mg/kg•d)	N	Mean ± SE				
		Body weight (g)	Spleen weight (mg)	Thymus weight (mg)	Spleen wt./ body wt. ($\times 10^{-3}$)	Thymus wt./ body wt. ($\times 10^{-3}$)
Cage control	10	230 ± 2	514 ± 10	295 ± 13	2.23 ± 0.04	1.28 ± 0.05
Vehicle control	10	226 ± 2	502 ± 10	272 ± 9	2.22 ± 0.04	1.21 ± 0.05
0.625	10	226 ± 2	494 ± 9	281 ± 14	2.18 ± 0.04	1.24 ± 0.06
1.25	10	229 ± 1	510 ± 9	294 ± 9	2.23 ± 0.03	1.28 ± 0.04
2.50	10	229 ± 1	516 ± 8	301 ± 9	2.25 ± 0.03	1.31 ± 0.03
5.0	10	228 ± 2	531 ± 8	293 ± 13	2.32 ± 0.03	1.28 ± 0.05
10.0	10	184 ± 4[a]	380 ± 19[a]	182 ± 25[a]	2.06 ± 0.07[a]	0.97 ± 0.13[a]

[a]Significant at $p < 0.01$, Dunnett's t-test versus cage control.

at this dosage was evident, since those animals also displayed reduced body weight and tremors which are characteristic of chlordecone intoxication. On the other hand, significant ($p < 0.01$) decreases in spleen and thymus weights were observed at all dosages of cyclophosphamide (Table 2). Significant decreases in spleen cellularity were associated with decreased spleen weights (data not shown). Significant body weight loss, however, was observed only at the two highest dosages of cyclophosphamide (i.e., 12 and 24 mg/kg•d), with 24 mg/kg•d being lethal for 7 of the 10 rats in this dosage group.

Table 3 shows that all three differentiated types of leukocytes were reduced in the highest chlordecone dosage group (10 mg/kg•d); however, only the decrease in circulating neutrophils was statistically significant ($p < 0.05$). The leukotoxic effect of cyclophosphamide is seen in Table 4.

TABLE 2. The Effect of Cyclophosphamide on Rat Lymphoid Organ and Body Weights

Treatment (mg/kg•d)	N	Mean ± SE				
		Body weight (g)	Spleen weight (mg)	Thymus weight (mg)	Spleen wt./ body wt. ($\times 10^{-3}$)	Thymus wt./ body wt. ($\times 10^{-3}$)
Cage control	10	232 ± 3	514 ± 7	302 ± 19	2.2 ± 0.03	1.3 ± 0.07
Vehicle control	10	232 ± 4	511 ± 12	282 ± 15	2.2 ± 0.04	1.2 ± 0.06
1.5	10	231 ± 4	452 ± 10[b]	244 ± 15[b]	1.9 ± 0.02[b]	1.1 ± 0.06[b]
3	10	229 ± 2	390 ± 6[b]	182 ± 7[b]	1.7 ± 0.03[b]	0.8 ± 0.03[b]
6	10	229 ± 3	328 ± 7[b]	82 ± 5[b]	1.4 ± 0.03[b]	0.4 ± 0.02[b]
12	10	213 ± 3[b]	269 ± 5[b]	52 ± 3[b]	1.3 ± 0.03[b]	0.2 ± 0.01[b]
24	3[a]	169 ± 7[b]	215 ± 21[b]	5 ± 4[b]	1.3 ± 0.10[b]	0.03 ± 0.02[b]

[a]Only 3 rats survived this dosage.
[b]Significant at $p < 0.01$, Dunnett's t-test versus cage control.

TABLE 3. The Effects of Chlordecone on Blood Leukocyte Counts

Treatment (mg/kg·d)	N	Mean ± SE			
		Leukocytes/ mm³	Lymphocytes/ mm³	Neutrophils/ mm³	Monocytes/ mm³
Cage control	10	5552 ± 128	4509 ± 334	928 ± 294	109 ± 11
Vehicle control	10	5984 ± 323	5274 ± 244	596 ± 119	113 ± 12
0.625	10	6294 ± 231	5564 ± 228	612 ± 86	118 ± 14
1.25	10	6754 ± 255	5861 ± 300	780 ± 84	113 ± 13
2.5	10	6568 ± 333	5758 ± 263	677 ± 146	113 ± 21
5.0	10	6055 ± 169	5444 ± 148	509 ± 83	102 ± 17
10.0	10	4768 ± 512	4397 ± 458	290 ± 57[a]	81 ± 14

[a]Significant at $p < 0.05$, Dunnett's t-test versus cage control.

Lymphocyte and total leukocyte counts were significantly ($p < 0.01$) reduced at all dosages of cyclophosphamide employed. A decrease in the number of neutrophils, however, was observed only at the two highest dosages (i.e., 12 and 24 mg/kg·d), while monocyte counts were significantly ($p < 0.01$) reduced at all but the lowest dosage (1.5 mg/kg·d). Monocytes were not observed in blood smears from rats surviving the highest dosage.

Tables 5 and 6 show the lymphoproliferative response data for optimal stimulating concentrations of the T-cell mitogens Con A (0.5 μg/culture) and PHA (0.5 μg/culture); the B-cell mitogen STM (4 μg/culture); and the T- and B-cell mitogen PWM (10 μg/culture). No clear dose-related pattern in mitogen responsiveness was observed for rats treated with chlordecone (Table 5). A significant ($p < 0.01$) increase in [^{3}H]TdH uptake was observed for splenocytes from the highest dosage group of chlordecone in

TABLE 4. The Effect of Cyclophosphamide on Blood Leukocyte Counts

Treatment (mg/kg·d)	N	Mean ± SE			
		Leukocytes/ mm³	Lymphocytes/ mm³	Neutrophils/ mm³	Monocytes/ mm³
Cage control	10	6390 ± 311	5696 ± 334	604 ± 100	90 ± 11
Vehicle control	10	6424 ± 388	5656 ± 323	663 ± 113	89 ± 12
1.5	10	4425 ± 243[a]	3744 ± 257[a]	525 ± 91	65 ± 10[a]
3	10	2990 ± 135[a]	2332 ± 108[a]	609 ± 84	49 ± 8[a]
6	10	1773 ± 72[a]	1320 ± 73[a]	368 ± 60	21 ± 3[a]
12	10	1205 ± 63[a]	953 ± 51[a]	228 ± 43[a]	19 ± 2[a]
24	3[b]	128 ±19 [a]	6 ± 19[a]	1 ± 1[a]	–

[a]Significant at $p < 0.01$, Dunnett's t-test versus cage control.
[b]Only 3 of 10 rats survived this dosage.

TABLE 5. The Effect of Chlordecone on the Lymphoproliferative Response of Rat Splenocytes

Treatment (mg/kg•d)	N	Mean cpm ($\times 10^{-3}$) ± SE				
		Cells only	Con A	PHA	PWM	STM
Cage control	10	2.3 ± 0.2	57.3 ± 2.2	41.9 ± 5.4	41.5 ± 2.4	18.3 ± 1.4
Vehicle control	10	2.5 ± 0.4	56.0 ± 2.8	43.0 ± 4.9	40.0 ± 3.5	19.8 ± 3.0
0.625	10	2.0 ± 0.3	63.5 ± 4.5	38.1 ± 4.9	35.9 ± 3.0	16.5 ± 1.9
1.25	10	1.9 ± 0.2	66.3 ± 5.6	31.4 ± 4.1	35.3 ± 3.7	14.5 ± 1.6
2.5	10	1.6 ± 0.2	60.0 ± 3.5	21.9 ± 1.2[a]	28.9 ± 2.5	11.8 ± 2.0
5.0	10	2.2 ± 0.2	60.5 ± 3.4	29.6 ± 3.4	38.3 ± 2.7	18.6 ± 2.8
10.0	10	6.6 ± 1.1[a]	31.5 ± 2.7[a]	43.1 ± 3.3	43.9 ± 2.1	30.4 ± 1.2[a]

[a]Significant at $p < 0.01$, Dunnett's t-test versus cage control.

the absence of mitogen and in the presence of the B-cell mitogen STM. In contrast, the response to the T-cell mitogen Con A was significantly ($p < 0.01$) suppressed in this dosage group. No difference in response to PHA or PWM was observed at the 10-mg/kg•d dosage; however, a significant reduction in [^{3}H]TdR uptake was observed for splenocytes cultured in the presence of PHA from the 2.5-mg/kg•d dosage group. It is interesting to note that compared to all other groups, the 2.5-mg/kg•d dosage group displayed minimum [^{3}H]TdR uptake, excluding Con A. The PHA response for this group, however, was the only significantly ($p < 0.01$) suppressed group. No other significant differences in mitogen responsiveness were seen.

A significant suppression of lymphoproliferation was observed for rats dosed with cyclophosphamide (Table 6). Only 5 groups of rats (i.e., cage and vehicle controls, 1.5, 3.0, and 6.0 mg/kg•d) were evaluated for mitogen responsiveness because of an insufficient number of spleen cells in the 12- and 24-mg/kg•d dosage groups. Significant ($p < 0.01$) reductions in [^{3}H]TdR uptake by spleen cells in the absence of mitogens were observed at dosages of 1.5, 3.0, and 6.0 mg/kg•d. Concomitantly, there was a significantly

TABLE 6. The Effect of Cyclophosphamide on the Lymphoproliferative Response of Rat Splenocytes

Treatment (mg/kg•d)	N	Mean cpm ($\times 10^{-3}$) ± SE				
		Cells only	Con A	PHA	PWM	STM
Cage control	10	1.6 ± 0.2	74.1 ± 5.2	43.2 ± 6.9	65.1 ± 2.7	11.9 ± 2.2
Vehicle control	10	1.6 ± 0.1	71.2 ± 3.7	56.4 ± 6.7	55.5 ± 1.9	13.9 ± 2.2
1.5	10	0.5 ± 0.1[b]	47.0 ± 5.8[b]	20.9 ± 2.3[b]	43.3 ± 5.3[b]	3.3 ± 0.6[b]
3	10	0.3 ± 0.03[b]	34.7 ± 7.6[b]	20.9 ± 4.0[b]	25.1 ± 6.8[b]	1.4 ± 0.4[b]
6	7[a]	0.2 ± 0.02[b]	27.0 ± 1.7[b]	18.8 ± 2.2[b]	10.3 ± 0.8[b]	0.8 ± 0.4[b]

[a]Insufficient spleen cells from 3 rats from this dosage group and all rats from the 12- and 24-mg/kg•d dosage groups to perform this assay.

[b]Significant at $p < 0.01$, Dunnett's t-test versus cage control.

TABLE 7. Natural Killer Cell Activity of Chlordecone-Dosed Rats Against W/Fu-G1 Target Cells

Treatment (mg/kg•d)	N	Mean % ^{51}Cr release ± SE for E:T ratio of		
		25:1	50:1	100:1
Cage control	10	4.5 ± 0.7	8.9 ± 0.9	10.8 ± 1.2
Vehicle control	10	4.6 ± 0.5	7.8 ± 0.9	8.4 ± 1.0
0.625	10	3.6 ± 0.8	7.1 ± 1.2	9.3 ± 1.5
1.25	10	3.9 ± 0.9	7.9 ± 1.1	9.1 ± 1.2
2.5	10	4.6 ± 0.8	8.3 ± 0.7	8.9 ± 1.0
5.0	10	3.9 ± 0.6	7.2 ± 1.0	7.9 ± 0.8
10.0	10	1.4 ± 0.5[a]	2.9 ± 0.5[a]	2.9 ± 0.5[a]

[a]Significant at $p < 0.01$, Dunnett's t-test versus cage control.

($p < 0.01$) lower uptake of [^{3}H]TdR by splenocytes cultured in the presence of all the mitogens tested.

The NK cell activity of splenocytes from chlordecone-dosed rats against the allogeneic W/Fu-G1 rat lymphoma target cell line and the xenogeneic YAC-1 mouse lymphoma target cell line is shown in Tables 7 and 8, respectively. Significant ($p < 0.01$) reductions in NK cell activity against both target cells were observed only at the highest dosage. Cyclophosphamide dosages of 6 and 12 mg/kg•d significantly ($p < 0.01$) suppressed NK cell activity against W/Fu-G1 (Table 9) and YAC-1 (Table 10) targets, respectively. There was essentially no change in NK activity at lower cyclophosphamide dosages when compared with controls. Rat NK cell activity against YAC-1 targets was consistently greater than that for W/Fu-G1 targets.

DISCUSSION

Alterations in the immune parameters examined were found only in rats receiving the highest dosage of chlordecone (10 mg/kg•d). The ob-

TABLE 8. Natural Killer Cell Activity of Chlordecone-Dosed Rats Against YAC-1 Target Cells

Treatment (mg/kg•d)	N	Mean % ^{51}Cr release ± SE for E:T ratio of		
		25:1	50:1	100:1
Cage control	10	9.0 ± 0.7	17.3 ± 1.0	22.1 ± 1.2
Vehicle control	10	9.1 ± 0.7	16.0 ± 0.8	19.8 ± 2.2
0.625	10	7.4 ± 0.7	14.3 ± 1.0	19.0 ± 1.3
1.25	10	7.9 ± 0.7	15.0 ± 1.0	19.7 ± 0.9
2.5	10	8.3 ± 0.7	15.9 ± 1.0	20.4 ± 1.5
5.0	10	7.7 ± 0.5	14.4 ± 0.8	17.5 ± 0.7
10.0	10	3.3 ± 0.5[a]	6.5 ± 0.7[a]	7.3 ± 1.0[a]

[a]Significant at $p < 0.01$, Dunnett's t-test versus cage control.

TABLE 9. The Effect of Cyclophosphamide on Natural Killer Cell Activity Against W/Fu-G1 Target Cells

Treatment (mg/kg·d)	N	Mean % ^{51}Cr release ± SE for E:T ratio of		
		25:1	50:1	100:1
Cage control	10	6.6 ± 0.8	9.9 ± 0.8	9.2 ± 1.0
Vehicle control	10	5.4 ± 0.5	9.5 ± 0.6	9.0 ± 0.6
1.5	10	6.0 ± 0.4	9.0 ± 0.6	8.9 ± 0.8
3	10	5.8 ± 0.6	8.2 ± 0.8	7.8 ± 0.9
6	10	2.1 ± 0.7[b]	2.2 ± 0.7[b]	0.9 ± 0.5[b]
12	8[a]	0.3 ± 0.5[b]	–	–

[a]Insufficient spleen cells from rats at this dosage to perform this assay at 50:1 and 100:1 ratios. Insufficient spleen cells from the 3 surviving rats at the 24-mg/kg dosage to perform this assay.
[b]Significant at $p < 0.01$, Dunnett's t-test versus cage control.

served effects of decreased lymphoid organ mass and cellularity, altered lymphoproliferative response, and decreased NK cell activity at this dosage were accompanied by a decrease in body weight. Rats at this dosage also displayed tremors that are characteristic of chlordecone intoxication (Gerhart et al., 1983). It appears therefore that the observed effects are due to a generalized intoxication of the rats at a chlordecone dosage of 10 mg/kg·d. A similar overt toxic response was observed in rats treated over a 10-d period by oral gavage with acrylamide at dosages ranging from 3.75 to 60 mg/kg·d. Decreased body weight and spleen and thymic weights were observed in the rats receiving the highest dosage only. Mitogen and NK responses were also depressed in this dosage group, in which 3 of the 10 rats died as a result of acrylamide treatment (unpublished results).

The generalized intoxication of rats with chlordecone may also have resulted in the induction of glucocorticoid release as part of a generalized stress response or as a result of a more specific effect upon the hypophyseal–

TABLE 10. The Effect of Cyclophosphamide on Natural Killer Cell Activity Against YAC-1 Target Cells

Treatment (mg/kg·d)	N	Mean % ^{51}Cr release ± SE for E:T ratio of		
		25:1	50:1	100:1
Cage control	10	12.5 ± 1.1	21.5 ± 1.0	24.0 ± 1.3
Vehicle control	10	11.1 ± 1.3	19.8 ± 1.5	23.1 ± 1.6
1.5	10	12.3 ± 0.5	21.0 ± 0.9	24.9 ± 1.3
3	10	12.9 ± 1.0	22.9 ± 1.3	25.8 ± 1.6
6	10	7.4 ± 1.0[a]	11.0 ± 1.2[a]	10.3 ± 1.0[a]
12	9[b]	2.2 ± 0.6[a]	3.2 ± 0.5[a]	2.9 ± 0.4[a]

[a]Significant at $p < 0.01$, Dunnett's t-test versus cage control.
[b]Insufficient spleen cells from 1 rat at this dosage and the 3 surviving rats at the 24-mg/kg dosage to perform this assay.

adrenal axis. Thymic involution, decreased responsiveness to Con A, and decreased NK cell activity can be produced by the release of glucocorticoids during a generalized stress response or by the administration of glucocorticoids (Selye, 1937; Monjan and Collector, 1977; Hockman and Cudkowicz, 1979; Smialowicz et al., 1983). Further evidence for a possible role of a stress-mediated response is the fact that only the 10-mg/kg•d dosage group was observed to have larger adrenal glands (data not shown). Hypertrophy of adrenal cortical and medullary cells was reported in Japanese quail fed chlordecone (Eroschenko and Wilson, 1975), and ultrastructural changes in cells of rats dosed with chlordecone were similar to those seen in animals subjected to acute stress (Baggett, et al., 1980). An increase in adrenal weight concomittant with a decrease in thymus weight is especially characteristic of a generalized stress response (Selye, 1937).

While the effects of glucocorticoids on lymphocyte function are not completely understood, the pattern of lymphoproliferation by spenocytes from rats in the highest chlordecone dosage group suggest that endogenous glucocorticoids may have played a role. From studies of mouse thymic lymphocytes, it is known that the majority of lymphocytes that are responsive to PHA are cortisone-resistant, while most Con A-responsive lymphocytes are cortisone sensitive (Adorini et al., 1976). This may explain why Con A, but not PHA, responses were suppressed at the highest dosage of chlordecone. Con A-responsive lymphocytes also comprise the bulk of suppressor T cells (Adorini et al., 1976). A stress-induced decrease in the Con A-responsive suppressor T-cell subpopulation may have allowed the enhanced proliferation of splenocytes from the 10-mg/kg•d dosage group in the absence of mitogen and in response to the B-cell mitogen STM. This might also help to explain the mitogen response data for the 2.5-mg/kg•d dosage group (Table 5). That is, at dosages greater than 2.5 mg/kg•d, the release of glucocorticoids associated with the stress response due to overt chlordecone toxicity may become the dominant factor by impairing Con A-sensitive suppressor cells, and freeing other cells from T-suppressor restraint. While this scenario is only speculative, it further suggests that a generalized stress reaction may be mediating the changes observed at this toxic dose of chlordecone. In any event, the data indicate that the treatment of rats with chlordecone under the conditions described here probably does not cause immunotoxicity directly, but rather the response most likely results from indirect effects as reported elsewhere (Vos, 1977). In light of the above discussion regarding stress and the immune system, it is noteworthy that no differences were observed for any of the parameters examined in the present studies between cage and vehicle controls. This indicates that under the conditions described for these studies, any stress that might be associated with handling and dosing rats is not reflected in altered responses.

To our knowledge, there are no published studies of the immunotoxic effects of in vivo exposure to chlordecone upon the functional integrity of

the immune system. In a recent review of the effects of pesticides on the immune system, no mention is made of any such studies with chlordecone (Street, 1981). In agreement with our results, with the exception of the high-dosage group, no change was reported in the peripheral leukocyte counts of rats following acute, subchronic, and chronic dosing with chlordecone (Larson et al., 1979). In this same study, however, an increase was observed in the spleen-to-body weight ratios of female rats fed 25 ppm chlordecone over a 2-yr period. This discrepancy between our results and those of Larson et al. (1979) may be due to the differences in the duration of treatment and/or to differences in the sexes studied. In an earlier in vivo study of the toxic effects of chlordecone exposure in the mouse, immunologic endpoints were not examined (Huber, 1965). Recently, Carmines et al. (1979) reported that in vitro exposure to chlordecone produced a dose-dependent inhibition of cellular proliferation, and caused a stimulation of respiration and a bimodal alteration (depression following enhancement) of the phagocytic activity in a macrophage-like cell line. These effects were suggested to be due to alterations in Ca utilization. Work by others also suggests that chlordecone inhibits Ca ion transport in the brain (End et al., 1979).

In contrast to chlordecone, 10-d oral dosing of Fischer-344 male rats with the potent immunosuppressive drug cyclophosphamide (Turk and Poulter, 1972; Jeejeebhoy, 1970) significantly affected all of the parameters examined at several dosage levels. We found that T and B lymphocytes were more sensitive to cyclophosphamide than were NK lymphocytes. Significant suppression of mitogen-stimulated lymphoproliferation was observed at the lowest dosage employed (1.5 mg/kg•d), while suppression of NK activity occurred at dosages of 6 mg/kg•d and above.

The results reported here are in agreement with a report which showed that a single oral dose of cyclophosphamide at 15 mg/kg significantly suppressed the mitogen response of rat peripheral blood lymphocytes to Con A (Noble and Norbury, 1983). Suppression of the lymphoproliferative response, in the present study, to the B-cell mitogen STM also is in agreement with work that has demonstrated that cyclophosphamide affects B-lymphocyte function (Turk and Poulter, 1972; Snippe et al., 1978; Dean et al., 1979).

The relative insensitivity of NK cell activity compared with the mitogen-stimulated lymphoproliferative response following cyclophosphamide dosing was somewhat surprising. Cyclophosphamide is known to suppress NK activity, albeit at much higher levels than were employed in the present study (Riccardi et al., 1981; Oehler and Herberman, 1978). We are unaware of any studies in rats in which a dose response to cyclophosphamide for NK cell activity had been performed. Our results indicate that a total oral dosage of 60 mg/kg over a 10-d period is sufficient to suppress significantly NK activity against both allogeneic W/Fu-G1 rat lymphoma cells and xenogeneic YAC-1 mouse lymphoma cells in rats. In an earlier study, a single

intraperitoneal injection of 125 mg cyclophosphamide/kg was not found to be very effective in suppressing NK activity in the rat (Oehler and Herberman, 1978). Our results therefore suggest that repeated dosing with cyclophosphamide may be more effective in suppressing NK activity than is a single dose, or that oral dosing is more effective than the intraperitoneal route.

It is also interesting to note that YAC-1 targets are more sensitive to rat NK-cell lysis than are W/Fu-G1 cells. However, significant alterations in NK-cell activity by either cyclophosphamide or chlordecone were detected at the same dosage using either cell as a target for NK lysis. These results suggest that either cell line can be used successfully to assess NK-cell activity in the rat.

A dosage of 12 mg/kg•d cyclophosphamide caused a significant decrement in body weight as well as all other measured parameters. While lymphoid organ weights, cell counts, and immune functions were significantly reduced, at the lower dosages, no change was observed in body weight. These results indicate that generalized weight loss is a poor predictor of a potential toxicant. Obviously, one must examine system-specific parameters in order to determine the potential for a toxicant to affect adversely the immune system. The assays employed in this study have been shown to be sensitive predictors of an immunotoxic effect. These assays correlate very well with in vivo immune parameters such as antibody responses, tumor models, delayed hypersensitivity responses, and infectivity models in animals exposed to a variety of immunotoxicants (Dean et al., 1979; Luster et al., 1981; Smialowicz et al., 1984, 1985). The immunologic assays employed in the work presented here are capable of detecting alterations caused by an agent with direct (specific) immunosuppressive action. These same parameters also enabled us to determine that chlordecone is not directly immunotoxic for T, B, and NK cells.

REFERENCES

Adorini, L., Ruco, L., Uccini, S., DeFranceshi, G. S., Baroni, C. D., and Doria, G. 1976. Biological effects of *Escherichia coli* lipopolysaccharide (LPS) *in vivo*. II. Selection in the mouse thymus of PHA- and Con A-responsive cells. *Immunology* 31:225–232.

Ahlquist, J. 1976. Endocrine influence on lymphatic organs, immune responses, inflammation and autoimmunity. *Acta Endocrinol.* 83(Supp. 206):3–136.

Anonymous. 1976. Report on carcinogenesis bioassay of technical grade chlordecone (Kepone). Carcinogenesis Program, Division of Cancer Cause and Prevention, National Cancer Institute, Bethesda, Md.

Baggett, J. McC., Thureson-Klein, A., and Klein, R. L. 1980. Effects of chlordecone on the adrenal medulla of the rat. *Toxicol. Appl. Pharmacol.* 52:312–322.

Baxter, C. S., Fish, L. A., and Bosh, J. A. 1981. Parallel orders of reactivity in murine cells of mezerein and phorbol esters toward the mixed-lymphocyte response and promotion of tumorigenesis. *Toxicol. Appl. Pharmacol.* 59:173–176.

Carlson, D. A., Konyha, K. D., Wheeler, W. B., Marshall, G. P., and Zoylskie, R. G. 1976. Mirex in the environment: Its degradation to Kepone and related compounds. *Science* 194:939–941.

Carmines, E. L., Carchman, R. A., and Borzelleca, J. F. 1979. Kepone: Cellular cites of action. *Toxicol. Appl. Pharmacol.* 49:543–550.

Chernoff, N. and Rogers, E. H. 1976. Fetal toxicity of Kepone in rats and mice. *Toxicol. Appl. Pharmacol.* 38:189–194.

Cohn, W. J., Blanke, R. V., Griffith, F. D., Jr., and Guzelian, P. S. 1976. Distribution and excretion of Kepone in humans. *Gastroenterology* 71:901.

Dean, J. H., Padarathsingh, M. L., Jerrells, T. R., Keys, L., Northing, J. W. 1979. Assessment of immunobiological effects induced by chemicals, drugs or food additives. II. Studies with cyclophosphamide. *Drug Chem. Toxicol.* 2:133–153.

Egle, J. L., Jr., Guzelian, P. S., and Borzelleca, J. F. 1979. Time course of the acute toxic effects of sublethal doses of chlordecone (Kepone). *Toxicol. Appl. Pharmacol.* 48:533–536.

End, D. W., Carchman, R. A., Ameen, R., and Dewey, W. L. 1979. Inhibition of rat brain mitochondrial calcium transport by chlordecone. *Toxicol. Appl. Pharmacol.* 51:189–196.

Eroschenko, V. P. and Mousa, M. A. 1979. Neonatal administration of insecticide chlordecone and its effects on the development of the reproductive tract in the female mouse. *Toxicol. Appl. Pharmacol.* 49:151–159.

Eroschenko, V. P., and Wilson, W. O. 1975. Cellular changes in the gonads, liver and adrenal gainds of Japanese quail as affected by the insecticide Kepone. *Toxicol. Appl. Pharmacol.* 31:491–504.

Gerhart, J. M., Hong, J. -S., and Tilson, H. A. 1983. Studies on the possible sites of chlordecone-induced tremor in rats. *Toxicol. Appl. Pharmacol.* 70:382–389.

Good, E. E., Ware, G. W., and Miller, D. F. 1965. Effects of insecticides on reproduction in the laboratory mouse. I. Kepone. *J. Econ. Entomol.* 58:754–757.

Hockman, P. S. and Cudkowicz, G. 1979. Suppression of natural cytotoxicity by spleen cells of hydrocortisone-treated mice. *J. Immunol.* 123:968–976.

Huber, J. J. 1965. Some physiological effects of the insecticide Kepone in the laboratory mouse. *Toxicol. Appl. Pharmacol.* 7:516–524.

Jeejeebhoy, H. F. 1970. The effect of heterologous antilymphocyte serum on lymphocytes of thymus and marrow origin. *J. Exp. Med.* 132:963–975.

Larson, P. S., Egle, J. L., Jr., Hennigar, G. R., Lane, R. W., and Borzelleca, J. F. 1979. Acute, subchronic, and chronic toxicity of chlordecone. *Toxicol. Appl. Pharmacol.* 48:29–41.

Luster, M. I., Faith, R. F., McLachland, J. A., and Clark, G. C. 1979. Effect of *in utero* exposure to diethylstilbestrol on the immune response in mice. *Toxicol. Appl. Pharmacol.* 15:441–450.

Luster, M. I., Dean, J. H., Boorman, G. A., Archer, D. L., Lauer, L., Lawson, L. D., Moore, J. A., and Wilson, R. E. 1981. The effects of orthophenylphenol, tris (2,3-dichloropropyl) phosphate, and cyclophosphamide on the immune system and host susceptibility of mice following subchronic exposure. *Toxicol. Appl. Pharmacol.* 15:441–450.

Metcalf, R. L., Kapoor, S. P., Lu, P. Y., Schuth, C. K., and Sherman, P. 1973. Model ecosystem studies of the environmental fate of six organochlorine pesticides. *Environ. Health Perspect.* 4:35–44.

McFarland, L. F. and Lacey, P. B. 1969. Physiologic and endocrinologic effects of the insecticide Kepone in the Japanese quail. *Toxicol. Appl. Pharmacol.* 15:441–450.

Monjan, A. A. and Collector, M. I. 1977. Stress-induced modulation of the immune response. *Science* 196:307–308.

Naber, E. C. and Ware, G. W. 1965. Effect of Kepone and mirex on reproductive performance in the laying hen. *Poult. Sci.* 44:875–880.

Noble, C. and Norbury, K. C. 1983. The differential sensitivity of rat peripheral blood T cells to immunosuppressants: Cyclophosphamide and dexamethasone. *J. Immunopharmacol.* 5:341–358.

Oehler, J. R. and Herberman, R. B. 1978. Natural cell-mediated cytotoxicity in rats. III. Effects of immunopharmacologic treatments on natural reactivity augmented by polyinosinic-polycytidylic acid. *Int. J. Cancer* 21:221–229.

Riccardi, C., Barlozzari, T., Santoni, A., Herberman, R. B., and Cesarini, C. 1981. Transfer to cyclophosphamide-treated mice of natural killer (NK) cells and *in vivo* natural reactivity against tumors. *J. Immunol.* 125:1284–1289.

Selye, H. 1937. Studies on adaptation. *Endocrinology* 21:169–188.

Seth, P. K., Agrawal, A. K., and Bondy, S. C. 1981. Biochemical changes in the brain consequent to dietary exposure of developing and mature rats to chlordecone (Kepone). *Toxicol. Appl. Pharmacol.* 59:262–267.

Slijivic, V. S. and Warr, G. W. 1973. Oestrogens and immunity. *Period. Biol.* 75:231–243.

Smialowicz, R. J., Rogers, R. R., Garner, R. J., Riddle, M. M., Luebke, R. W., and Rowe, D. G. 1983. Microwaves (2,450 MHz) suppress murine natural killer cell activity. *Bioelectromagnetics* 4: 371–381.

Smialowicz, R. J., Rogers, R. R., Riddle, M. M., and Stott, G. A. 1984. Immunologic effects of nickel: I. Suppression of cellular and humoral immunity. *Environ. Res.* 33:413–427.

Smialowicz, R. J., Rogers, R. R., Riddle, M. M., Garner, R. J., Rowe, D. G., and Luebke, R. W. 1985. Immunologic effects of nickel: II. Suppression of natural killer cell activity. *Environ. Res.* 36: 70–76.

Snippe, H., Merchant, B., Johannessen, L., and Inman, J. K. 1978. Effects of cyclophosphamide on the *in vivo* response of outbred athymic (nude) mice to a thymus-independent antigen (DNP-AGG-Ficoll). *Immunology* 35:1009–1015.

Stevens, R. H. and Cole, D. A. 1982. Identification of carcinogens by measurement of cell-mediated immunity. II. Assay specificity. *Environ. Res.* 28:67–76.

Street, J. C. 1981. Pesticides and the immune system. In *CRC Immunologic Considerations in Toxicology*, ed. R. P. Sharma, vol. 1, pp. 45–66. Boca Raton, Fla.: CRC.

Taylor, J. R., Selhorst, J. B., Houff, S. A., and Martinez, J. 1978. Chlordecone intoxication in man. *Neurology* 28:626–631.

Turk, J. L. and Poulter, L. W. 1972. Selective depletion of lymphoid tissue by cyclophosphamide. *Clin. Exp. Immunol.* 10:285–296.

Vos, J. G. 1977. Immune suppression as related to toxicology. *CRC Crit. Rev. Toxicol.* 5:67–101.

Received September 28, 1984
Accepted January 30, 1985